methods; guanidinium thiocyanate acid-phenol based extraction methods; guanidinium thiocyanate acid based extraction methods; methods using centrifugation through cesium chloride or similar gradients; phenol-chloroform based extraction methods; or other commercially available RNA extraction methods. In this aspect of the invention, RNA is extracted from plasma, serum, or other bodily fluid. In other aspects of the invention, extraction may alternatively be performed using probes that specifically hybridize to a particular RNA.

In preferred embodiments of the inventive methods, EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, hnRNP A2/B1 RNA or any combination thereof, or more preferably cDNA derived therefrom is amplified using an *in vitro* amplification method such as reverse transcriptase polymerase chain reaction (RT-PCR); ligase chain reaction; DNA signal amplification; amplifiable RNA reporters; Q-beta replication; transcription-based amplification; isothermal nucleic acid sequence based amplification; self-sustained sequence replication assays; boomerang DNA amplification; strand displacement activation; cycling probe technology; or any combination or variation thereof.

In preferred embodiments of the inventive methods, amplification products of EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, hnRNP A2/B1 RNA or any combination thereof, or more preferably cDNA produced therefrom, are detected using a detection method such as gel electrophoresis; capillary electrophoresis; conventional enzyme-linked immunosorbent assay (ELISA) or modifications thereof, such as amplification using biotinylated or otherwise modified primers; nucleic acid hybridization using specific labeled probes, such as fluorescent-, radioisotope-, or chromogenically-labeled probes; Southern blot analysis; Northern blot analysis; electrochemiluminescence; laser-induced fluorescence; reverse dot blot detection; and high-performance liquid chromatography.

In particularly preferred embodiments of the inventive methods, RNA is converted to cDNA using reverse transcriptase following extraction of RNA from a bodily fluid and prior to amplification.

The methods of the invention are advantageously used for providing a diagnosis of, or as a predictive indicator for determining risk for a human of developing a proliferative, premalignant, neoplastic, or malignant disease comprising or characterized by cells expressing EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, hnRNP A2/B1 RNA or any combination thereof.

The methods of the invention are particularly useful for providing a diagnosis of or for identifying in animals, particularly humans, who are at risk for developing or who have developed malignancy or premalignancy of cells comprising epithelial tissues. Most preferably, malignant or premalignant diseases, conditions, or disorders advantageously detected or diagnosed using the methods of the invention are diseases or disorders of breast, ovaries, lung, cervix, colorectal, stomach, pancreas, bladder, endometrium, kidney, head and neck, and esophageal cancers, and premalignancies and carcinoma *in-situ* such as bronchial dysplasia, atypical hyperplasia of the breast, ductal carcinoma in-situ, colorectal adenoma, atypical endometrial hyperplasia, and Barrett's esophagus.

In certain preferred embodiments of the methods of the invention, EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, hnRNP A2/B1 RNA or combinations thereof, or cDNA derived therefrom is amplified in a quantitative manner, thereby enabling quantitative comparison of said RNA present in a bodily fluid such as blood plasma or serum from a human. In these embodiments, the amount of extracellular EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, or hnRNP A2/B1 RNA or combinations thereof are detected in an individual and

compared with a range of amounts of said extracellular RNA detected in the bodily fluid in a plurality of humans known to have a premalignant or malignant disease, or known to be free from a premalignant or malignant disease.

The invention further provides methods for identifying individuals having an EGF-, EGFr-, her-2/neu-, c-myc-, or hnRNP A2/B1-expressing malignancy or premalignancy, or a malignancy expressing any combination of said RNAs, thereby permitting rational, informed treatment options to be used for making therapeutic decisions. In particular, the methods of the invention are useful in identifying individuals having a premalignancy or malignancy that would benefit from a therapy directed at cells and tissues that express EGF, EGFr, her-2/neu-, c-myc-, or hnRNP A2/B1, such as monoclonal antibody therapy, anti-sense therapy, and vaccine therapy.

Another advantage of the use of the methods of the invention is that the methods can produce markers for assessing the adequacy of anticancer therapies such as surgical intervention, chemotherapy, biotherapy such as monoclonal antibody therapy or vaccines, anti-angiogenic therapy, and radiation therapy, and is also useful for determining whether additional or more advanced therapy is required. The invention therefore provides methods for developing a prognosis in such patients.

The methods of the invention also permit identification or analysis of EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, hnRNP A2/B1 RNA or any combination thereof, either qualitatively or quantitatively, in the blood or other bodily fluid of an animal, most preferably a human that has completed therapy, as an early indicator of relapsed cancer, impending relapse, or treatment failure.